



# Survey for Yellow Mosaic Disease Occurring on Blackgram [*Vigna mungo* (L.) Hepper] and Weed Hosts in Four Major Blackgram Growing Districts of Andhra Pradesh and Characterization of Associated Viruses using Coat Protein Gene

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## ABSTRACT

**Background:** Yellow mosaic disease (YMD) caused by *Yellow mosaic virus* is one of the major constraints in the pulse production in Andhra Pradesh (A.P.) due to fast evolution of strains, like *Mungbean yellow mosaic India virus* (MYMIV). Keeping this in view, a survey was undertaken in the major blackgram growing districts of A.P. to know the YMD incidence in blackgram and weed hosts and were characterized based on genetic features by comparing with other YMV isolates from different hosts and locations across the world.

**Methods:** Roving survey was conducted during *rabi* 2019-20 in major blackgram growing districts of A.P. viz., Krishna, Guntur, West Godavari and Prakasam districts for YMD incidence. Blackgram plants showing characteristic symptoms were collected as representative samples from each mandal along with the suspected weed plants and were subjected to amplification using coat protein (CP) specific primers followed by molecular characterization. Phylogenetic tree for coat protein (CP) gene was constructed using aligned sequences with 1000 bootstrap replicates following neighbor-joining phylogeny.

**Result:** Out of the four districts surveyed, the highest disease incidence was recorded at Machavaram village of Prakasam district (43.22%), whereas least disease incidence was recorded at Chinaganjam village of Prakasam district (2.4%). Six weeds viz., *Ageratum conyzoides*, *Amaranthus viridis*, *Parthenium hysterophorus*, *Vigna trilobata*, *Abelmoschus moschatus*, *Desmodium laxiflorum* have showed positive result in PCR amplification with MYMIV specific coat protein primers. Four isolates from blackgram samples and two from weed plants shared 94.85 to 99.58% nucleotide identity among themselves.

**Key words:** Blackgram, Coat protein gene, MYMIV, Survey, Yellow mosaic.

## INTRODUCTION

Blackgram is one of the important pulse crops grown throughout India which is consumed in the form of 'dal' (whole or split, husked and un-husked) or perched, its high value of lysine makes it an excellent complement to rice in terms of balanced human nutrition. India is one of the major producers of blackgram cultivated in 349.91 lakh ha with a production of 232.41 lakh tonnes in 2017 (Directorate of Economics and Statistics, M/A, GoI.). In India, during *kharif* 2019-20, area covered under blackgram is 37.52 lakh ha. with the major contributions from Madhya Pradesh (16.50 lakh ha), Uttar Pradesh (7.01 lakh ha), Rajasthan (4.56 lakh ha), Maharashtra (2.87 lakh ha), Karnataka (0.687 lakh ha) and Andhra Pradesh (0.11 lakh ha) (Blackgram Outlook Agricultural Market Intelligence Centre, PJTSAU, 2019). In Andhra Pradesh, the majority of the area is under Krishna, Guntur, West Godavari and Prakasam districts covering an area of 2.03 lakh ha. out of 3.18 lakh ha with a production of 2.35 lakh tonnes in which major area is covered in Krishna, Guntur, West Godavari and Prakasam districts. The area under blackgram cultivation is being reduced from 4.55 lakh hectares in 2015-16 to 3.18 lakh ha in 2018-19 (Statistical Abstract Andhra Pradesh 2019). The decline in the

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production was mainly due to biotic (viral and fungal diseases) and abiotic factors.

Among the viral diseases, YMD is one of the major constraints in the pulse production. It was reported that YMD was caused by four viruses - MYMV (*Mungbean yellow mosaic virus*), MYMIV (*Mungbean yellow mosaic india virus*), HgYMV (*Horsegram yellow mosaic virus*) and DoYMV (*Dolichus yellow mosaic virus*) across southern Asia which are collectively known as *Yellow mosaic viruses* (YMV's) (Qazi *et al.* 2007). In general, MYMV was the major isolate infecting pulse crops in Western and Southern India, Thailand and Indonesia whereas MYMIV isolate in Central, Eastern, Northern India, Pakistan, Bangladesh, Nepal and Vietnam (Malathi and John, 2009). However, first report of MYMIV from Andhra Pradesh was reported by Reddy *et al.* (2015).

Survey was undertaken in major blackgram growing districts of Andhra Pradesh to study the incidence of YMD in blackgram and weeds. The collected samples were subjected to molecular characterization of coat protein (CP) in blackgram and suspected weed isolates and compared with other YMV's isolates occurring on different hosts and locations.

## MATERIALS AND METHODS

Field and molecular lab studies were conducted in Department of Plant Pathology, Agricultural College, Bapatla and a part of molecular work was conducted at Plant Virology Lab, National Research Centre for Banana (NRCB), Tiruchirapalli.

### Survey

The survey was carried out to have first hand information on the occurrence of YMD in blackgram and weed hosts during *rabi* 2019-2020 in major blackgram growing districts of Andhra Pradesh viz., Krishna, Guntur, West Godavari and Prakasam districts. In each district, three mandals and in each mandal three villages and three fields from each village were surveyed. Five locations, i.e., at four corners of the field and one at the centre and in each location of one sq. m, incidence of YMD was recorded based on the symptoms. Disease incidence was calculated by using the formula given below. Samples of infected blackgram plants and suspected weed hosts were collected in plastic bags and brought to the laboratory for further analysis.

Data pertaining to the variety sown, predominant weed species, preceding/succeeding crop, surrounding crop was also recorded during the survey (Table1).

Per cent disease incidence =

$$\frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

### Molecular characterization of CP gene

#### DNA extraction

The total genomic DNA was isolated from the representative sample from each mandal (12 samples) and weed hosts

(15 samples) by using a protocol given by Rouhibakhsh *et al.* (2008) with slight modification.

#### PCR

The genomic DNA was subjected to PCR with coat protein specific primers (Table 2). The PCR amplifications were carried out in thermal cycler (Eppendorf master cycler gradient). The components of the reaction mixture were 1 µl of genomic DNA (50 ng), 0.5 µl each of 10 pm forward and reverse primers, 2.5 µl of 10X PCR buffer, 1.5 µl of 25 mM MgCl<sub>2</sub>, 0.5 µl of 10 mM dNTPs, 0.5 µl of Taq DNA polymerase (5U/µl) in nuclease free water to make up final volume up to 25 µl. (All the above reaction components were procured from Thermo scientific company). PCR profiles for different primers used are mentioned in the (Table 3). Subsequently, PCR product was run on one per cent agarose gel for an hour at 80 volts.

Each representative YMV isolate showing the highest disease incidence in that corresponding district were selected. YMV-KR (Krishna), YMV-GN (Guntur), YMV-WG (West Godavari) and YMV-PR (Prakasam) and two isolates from weed hosts YMV-ABEL (*Abelmoschus moschatus*), YMV-DES (*Desmodium laxiflorum*) were used for molecular characterization. The total genomic DNA isolated was amplified with MYMIV specific primers (~1065 bp) and the amplified products were outsourced to sequencing facility at Bangalore (Eurofins Genomics Bangalore India). Low quality nucleotide sequences at both the ends were trimmed by Chromas (version 2.6.6) and the trimmed sequences were assembled using Bioedit (version 7.0.5.3). Sequence identities among the isolates were obtained using Species Demarcation Tool (SDTv1.2) (Muhire *et al.*, 2014). Assembled sequence of partial CP gene was evaluated using BLASTN (www.ncbi.nlm.nih.gov) to know the identity and homology with other reported YMV isolates. For further analysis, reference sequences of CP gene sequences of YMV available in database were downloaded from NCBI website. Partial CP gene sequences of YMV were compared with seventeen reported YMV isolates from different parts of the world infecting various pulses and ToLCNdV is taken as an out group member. All the sequences were aligned using MUSCLE algorithm of MEGAX (version 10.1.1). The phylogenetic tree for CP gene was constructed using aligned sequences with 1000 bootstrap replicates following neighbor-joining phylogeny of MEGAX (Sudhir *et al.*, 2018).

## RESULTS AND DISCUSSION

### Roving survey

Survey was undertaken in four major blackgram growing districts of Andhra Pradesh (12 mandals and 36 villages). Blackgram crop was in the mid-vegetative stage to pod formation stage across the surveyed fields. Plants showing the symptoms of YMD like general yellowing, mosaic pattern, stunted growth of the plant and curved pods were recorded. To confirm the presence of YMV, representative sample from each surveyed mandal was subjected to PCR using MYMV

and MYMIV specific primers, where in, all samples were tested positive for MYMIV with an amplicon length of ~1065 bp (Fig 1).

The survey results indicate that there was a significant difference in the per cent disease incidence of YMD during

rabi 2019-20 in different districts of Andhra Pradesh ranging from 2.43% (Chinaganjam village of Ongole mandal in Prakasam district) to 43.22% (Machavaram village of Naguluppapadu mandal in Prakasam district) (Table 1). High disease incidence recorded in some villages of

**Table 1:** Incidence of yellow mosaic disease in major blackgram growing districts of Andhra Pradesh.

District	Mandal	Village	Variety sown	Predominant weed species	Surro- -unding crop	Preceding/ Succeeding crop	Disease -inci dence	
Krishna	Kankipadu	Kunderu	LBG-752	<i>Trichodesma indicum</i> , <i>Acalypha indica</i>	BG	R/R	20.84	
		Nepalle	LBG-752	<i>Commelina benghalensis</i> , <i>Vigna trilobata</i>	BG and GG	R/R	23.24	
		Prodduturu	Nandini	<i>Digeria muricata</i> , <i>Acalypha indica</i>	BG and SC	R/R	8.33	
	Vuyyuru	Akunuru	GBG-40	<i>Chrozophora rottleri</i> , <i>Cleome viscosa</i> .	BG	R/R	26.48	
		Potlapadu	LBG-752	<i>Commelina benghalensis</i> , <i>Ageratum conyzoides</i> .	BG and GG	R/R	36.2	
	Pamaruru	Kadavakollu	LBG-752	<i>Euphorbia hirta</i> , <i>Croton bonpladianu</i> .	BG	R/R	18.45	
		Pamaruru	GBG-40	<i>Acalypha indica</i> , <i>Cleome viscosa</i>	BG	R/R	18.66	
		Komaravole	Unknown	<i>Euphorbia geniculata</i>	BG	R/R	9.82	
		Kanumuru	Nandini	<i>Parthenium hysterophorus</i> , <i>Trichodesma indica</i>	BG and MZ	R/R	22.64	
	Guntur	Ponnuru	Sitaramapuram	LBG-752	<i>Commelina benghalensis</i>	BG	R/R	16.97
Upparapalem			LBG-752	<i>Xanthium strumarium</i>	MZ and BG	R/R	4.31	
Kakamanu		Kasukarru	Unknown	<i>Chrozophora rottleri</i> , <i>Ageratum conyzoides</i>	SG and BG	R/R	17.81	
		Pandrapadu	Unknown	<i>Euphorbia geniculata</i> , <i>Desmodium laxiflorum</i>	CH and BG	R/R	12.17	
		Chinalingaya palem	Unknown	<i>Ageratum conizoidis</i> , <i>Parthenium hyserophorus</i>	BG	R/R	31.38	
Vatticherukuru		Valluru	PU-31	<i>Cleome viscosa</i>	BG and MZ	R/R	9.78	
		Vatticherukuru	Local variety	<i>Croton bonpladianum</i> , <i>Acalypha indica</i>	GG and BG	R/R	14.5	
		Mutlur	Unknown	<i>Ageratum conizoidis</i> , <i>Digera arvensis</i>	BG and MZ	R/R	16.29	
West Godavari		Eluru	Kornepadu	PU-31	<i>Chrozophora rottleri</i>	BG and SG	R/R	8.02
			Komadavole	Unknown	<i>Parthenium hysterophorus</i> , <i>Cleome viscosa</i>	BG and MZ	R/R	26.44
	Denduluru	Sreeparru	LBG-752	<i>Commelina benghalensis</i>	BG and MZ	R/R	12.86	
		Chataparru	Unknown (Polish)	<i>Trichodesama indica</i>	BG and GG	R/R	14.38	
		Kovvali	PU-31	<i>Vigna trilobata</i> , <i>Euphorbia geniculata</i>	BG and CP	R/R	18.62	
	Pedapadu	Pothunuru	LBG-752	<i>Cleome viscosa</i> , <i>Ageratum conyzoides</i>	BG and GG	R/R	30.26	
		Gudigunta	Unknown	<i>Trichodesma indica</i> , <i>Amaranthus viridis</i>	BG	U	22.47	
		Vatluru	LBG-752	<i>Commelina benghalensis</i> , <i>Cleome viscosa</i>	BG and GG	R/R	8.64	
	Prakasam	Nagulup palapadu	Kothuru	PU-31	<i>Euphorbia hirta</i>	BG and MZ	R/R	16.48
			Naidugudem	Unknown	<i>Trichodesma indica</i>	BG and GG	U	18.49
Obannapalem			Unknown	<i>Digera muricata</i> , <i>Abelmoscus moschatus</i>	BG and GN	BG	29.88	
Ongole		Machavaram	LBG-752	<i>Acalypha indica</i> , <i>Ageratum conyzoides</i>	BG and GG	R/R	43.22	
		Ammanabrolu	LBG-752	<i>Amaranthus viridis</i> , <i>Croton bonpladianum</i>	BG and MZ	GN	32.00	
		Chekurupadu	Unknown	<i>Vigna trilobata</i> , <i>Cucumis trigonus</i>	BG and GG	BG	26.32	
Chirala		Tanguturu	TBG-104	<i>Digera arvensis</i>	BG and MZ	R/R	16.24	
		Chinaganjam	PU-31	<i>Acalypha indica</i> , <i>Cleome viscosa</i>	GN and BG	GN	2.43	
		Pullayapalem	Unknown	<i>Digera arvensis</i> , <i>Parthenium hysterophorus</i>	BG	U	24.6	
		Gavinivaripalm	LBG-623	<i>Desmodium laxiflorum</i> , <i>Cleome viscosa</i>	BG and GN	BG	14.24	
	Ipurupalem	Unknown	<i>Cleome viscosa</i> , <i>Vigna trilobata</i>	BG and GN	GN	18.42		

BG: Blackgram, GG: Greengram, R: Rice, SC: Sugarcane, MZ: Maize, SG: Sorghum, CH: Chilli, CP: Cowpea, GN: Groundnut, U: Unknown.

Prakasam district might be attributed to the cultivation of blackgram as preceding crop that might have resulted in higher whitefly population. Further, large scale cultivation of susceptible local varieties could also be ascribed to high disease incidence (Archith *et al.*, 2017). Prevalence of YMV susceptible weed hosts in Prakasam district might have helped in the perpetuation of the virus during offseason. Earlier studies in Koppal district of Karnataka emphasized the role of favourable weather conditions for biological development of whitefly for horizontal spread of virus during summer months and the role of weed hosts in perpetuation of YMD (Meghashree *et al.*, 2017).

Mean disease incidences of major blackgram growing districts of Andhra Pradesh viz., Krishna, Guntur, West Godavari and Prakasam districts were 20.51, 14.58, 18.73 and 23.03% respectively and the mean incidence among

all the surveyed districts was 19.21% with the lowest incidence in Guntur district (14.58%) followed by West Godavari (18.73%), Krishna (20.15%) and Prakasam (23.03%). The lowest disease incidence in Guntur, West Godavari and Krishna district might be ascribed to the rice fallow pulse cropping system with concomitant sowing dates and consequently less whitefly population.

Panduranga *et al.* (2012) surveyed Warangal and Khammam districts of Telangana and reported that the variation in YMD incidence was due to variation in sowing time of the crop and prevalence of different weeds which have contributed to the population dynamics of whitefly and subsequent incidence of YMD.

Similar studies done in different agro climatic zones of Karnataka (Jayappa *et al.*, 2017) reported the highest incidence of YMD on sole crop of mungbean compared to

**Table 2:** Primers used in the present study.

Description	Sequence	Amplicon size	Reference
MYMIV-CP-FP	5'ATG GAT TCC GGT GCA TGT TG 3'	~1065 bp	Agnihotri <i>et al.</i> , 2019
MYMIV-CP-RP	5'GAC TTC TGG GAT GAT CTT ATC GA 3'		
MYMV-CP-FP	5'ACA CGA GCT CCT CTA CCC CGA TAT CGA ATG 3'	~750 bp	Islam <i>et al.</i> , 2012
MYMV-CP-RP	5'ACA CGG ATC CGT TGC ATA CAC AGG ATT TG 3'		
MYMV-CP-FP	5'ATG GGT TCC GTT GTA TGC TTG 3'	~1000 bp	Prema and Rangaswamy, 2018
MYMV-CP-RP	5'GGC GTC ATT AGC ATA GGC AAT 3'		

**Table 3:** PCR profiles for YMV's amplification.

Steps in PCR	Temperature		
	1 <sup>st</sup> set of primers	2 <sup>nd</sup> set of primers	3 <sup>rd</sup> set of primers
Initial denaturation	94°C for 3 min	95°C for 5 min	94°C for 2 min
Denaturation	94°C for 30 sec	94°C for 1 min	94°C for 1 min
Annealing	50°C for 30 sec	58°C for 1 min	55°C for 2 min
Primer extension	72°C for 1 min	72°C for 30 sec	72°C for 3 min
Final extension	72°C for 5 min	72°C for 5 min	72°C for 5 min
Storage	Infinite hold at 4°C	Infinite hold at 4°C	Infinite hold at 4°C

**Table 4:** Weed hosts collected in the present study.

Weed	Virus detected	Symptoms
<i>Abelmoschus moschatus</i>	MYMIV	Yellow vein
<i>Acalypha indica</i>	-	Yellow and green patches
<i>Ageratum conyzoides</i>	MYMIV	Yellow vein
<i>Amaranthus viridis</i>	MYMIV	Dot like yellow spots
<i>Chrozophora rotlerii</i>	-	Yellow mosaic
<i>Cleome viscosa</i>	-	Mild yellowing at leaf tip
<i>Commelina benghalensis</i>	-	Yellow and green patches, Yellowing with green veins
<i>Croton bonplandianum</i>	-	Yellow spots
<i>Cucumis trigonus</i>	-	Mild yellowing in interveinal region
<i>Desmodium laxiflorum</i>	MYMIV	Yellow vein and Apical leaf curling and leaf crinkling
<i>Digera arvensis</i>	-	Complete yellowing
<i>Euphorbia geniculata</i>	-	Mild yellow patches
<i>Parthenium hysterophorus</i>	MYMIV	Yellowing of leaf tip
<i>Trichodesma indicum</i>	-	Mild yellowing and leaf curling
<i>Vigna trilobata</i>	MYMIV	Yellow vein and yellow mosaic

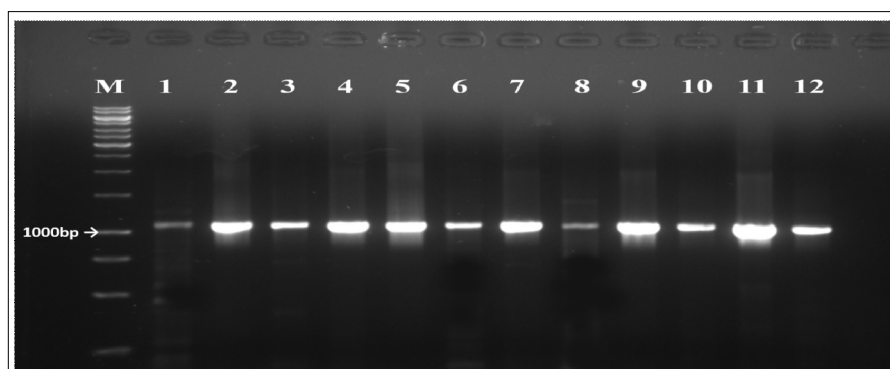
intercrop and established a positive correlation between YMD incidence and vector population. Manjunatha *et al.* (2013) reported the prevalence of B Biotype whitefly (*Bemisia tabaci*) and large-scale cultivation of susceptible varieties led to the increase in the incidence of YMD in Southern Karnataka. Salam *et al.* (2011) surveyed Dharwad, Gadag, Haveri, Gulbarga and Bidar districts of Karnataka and reported the highest disease incidence of YMD in mungbean in Bidar district and the variation in disease incidence over locations was attributed to the varied population dynamics of whitefly as influenced by temperature and relative humidity.

#### Weed hosts

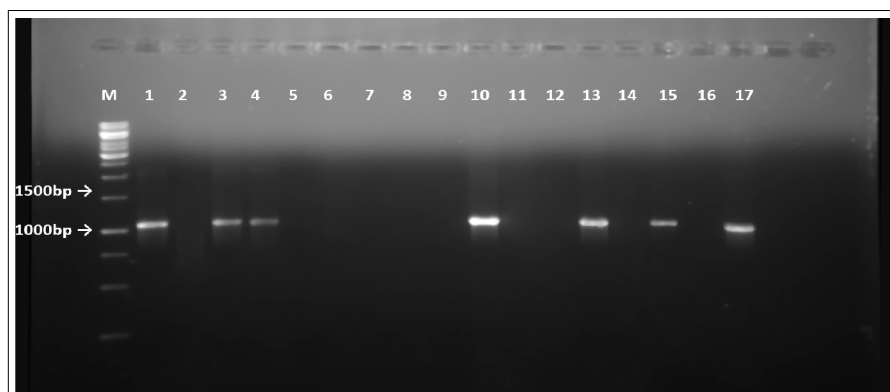
During survey, suspected weeds were identified, varied symptoms were observed (Table 4) and samples were collected for conformational studies using specific primers. Out of 15 weed species, six weeds viz., *Ageratum conyzoides*, *Amaranthus viridis*, *Parthenium hysterophorus*, *Vigna trilobata*, *Abelmoschus moschatus*, *Desmodium laxiflorum* were tested positive for MYMIV showing an amplicon length of 4 1065 bp (Fig 2). None of the weed species was tested

positive for MYMV. *Begomovirus* species cause numerous diseases in cultivated crops and weeds of the families Cucurbitaceae, Solanaceae, Fabaceae and Malvaceae which pose a threat to cultivation in different parts of India (Varma and Malathi, 2003).

Naimuddin *et al.* (2014) reported *Ageratum conyzoides*, a common weed growing throughout the year, as a new host for MYMIV and concluded that prevalence of this weed act as an important source of primary inoculum and is responsible for recurrence of yellow mosaic disease in grain legumes in Northern India. Bhanu *et al.* (2015) reported different weeds viz., *Ageratum conyzoides*, *Amaranthus viridis*, *Parthenium hysterophorus* and *Vigna trilobata* as alternate hosts by detecting them using MYMV specific primers whereas, in the present study MYMIV was noticed in the same weeds which emphasize the distribution of new species of YMD in Andhra Pradesh. Marabi *et al.* (2017) suspected ten weeds as potential weed hosts for YMV based on the visual symptoms but only four viz., *Ageratum conyzoides*, *Vigna trilobata*, *Corchorus olitorius* and *Alternanthera sessilis* were found to be true alternate hosts of MYMIV when tested using MYMIV specific markers. Cross

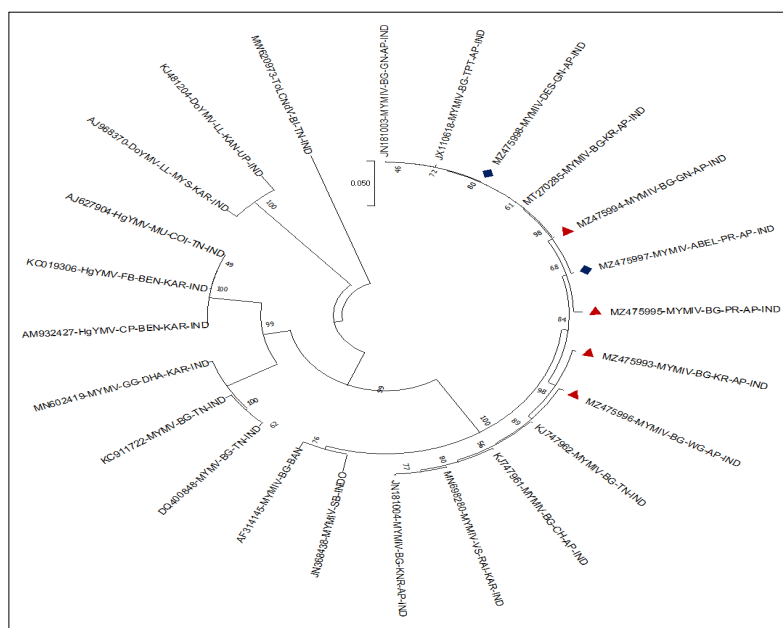


**Fig 1:** PCR amplification of Coat protein (CP) gene of MYMIV in blackgram isolates collected from different locations in Andhra Pradesh. M= 1 kb DNA ladder; Lane 1-12: (1- Kankipadu, 2- Vuyyuru, 3- Pamarru, 4- Ponnuru, 5- Kakamanu, 6- Vatticherukuru, 7- Eluru, 8- Denduluru, 9- Pedapadu, 10- Naguluppapadu, 11- Ongole, 12- Chirala).



**Fig 2:** PCR amplification of Coat protein (CP) gene of MYMIV in different weed species collected from different locations in Andhra Pradesh. M =1 kb DNA ladder; Lane 1-17: (1- *Abelmoschus moschatus*, 2- *Acalypha indica*, 3- *Ageratum conyzoides*, 4- *Amaranthus viridis*, 5- *Chrozophora rottleri*, 6- *Cleome viscosa*, 7- *Commelinabenghalensis*, 8- *Croton bonplandianum*, 9- *Cucumis trigonus*, 10- *Desmodium laxiflorum*, 11- *Digera arvensis*, 12- *Euphorbia geniculata*, 13- *Parthenium hysterophorus*, 14- *Trichodesma indicum*, 15- *Vigna trilobata*, 16- Negative control, 17- Positive control).





**Fig 3:** Phylogenetic comparison based on nucleotide sequences of coat protein (CP) gene of MYMIV isolates of present study with previously reported YMV isolates from India and other countries.

1. Each sequence is labelled with the NCBI accession number followed by Causal organism, Host and Location.
2. Isolates of present study in blackgram are highlighted with triangle mark and in weed hosts are highlighted with rhomboidal mark.

inoculation studies conducted by Deepa *et al.* (2017) using the insect vector whitefly (*Bemisia tabaci*) could not transmit the YMD to *Parthenium hysterophorus* concluding the weed as non host for MYMIV. In the present study, 15 weed hosts were suspected positive for YMD based on visual symptoms, of which six were found positive for MYMIV. Hence, molecular confirmation of the YMD suspected samples with MYMIV specific molecular markers could be a reliable technique to detect alternative hosts of MYMIV.

#### Molecular characterization of CP gene of Isolates

The representative YMD isolates, were sequenced and submitted to NCBI with the following accession numbers, YMV-KR (MZ475993), YMV-GN (MZ475994), YMV-WG (MZ475996), YMV-PR (MZ475995), YMV-ABEL (MZ475997) and YMV-DES (MZ475998).

A phylogenetic dendrogram based on an alignment of coat protein gene sequences of six isolates under the study with sequences available in NCBI database (Fig 3) revealed that all the test isolates clustered into MYMIV group and formed a separate clad with other species of the begomoviruses *i.e.*, MYMV, HgYMV, DoYMV. All the test isolates showed a unique feature of close clustering with MYMIV isolates originated from South India and distantly related with the foreign isolates (Indonesia and Bangladesh). The isolates YMV-GN, YMV-PR, YMV-DES, YMV-ABEL formed a closed clustering with Andhra Pradesh isolates from Guntur (JN181003), Tirupati (JX110618) and Krishna (MT270285) whereas, the isolates YMV-KR and YMV-WG formed a close clustering with the isolates from Andhra Pradesh (KJ747961, JN181004), Tamil Nadu (KJ747962)

and Karnataka (MN698280). The CP gene is known to be highly conserved gene in the family *Geminiviridae* which effectively predicts discrete strains, species and taxonomic lineage of begomoviruses that has been accepted by ICTV as desirable marker for virus identity when a full-length genomic sequence is not available (Rybicki 1998). Coat protein genes have traditionally proven useful for plant virus identification and classification (Mayo and Pringle 1997). According to these guidelines, the present isolates from crop and weed hosts *i.e.*, YMV-KR, YMV-GN, YMV-PR, YMV-WG, YMV-ABEL, YMV-DES belongs to MYMIV. It is clear from the above results that six isolates causing YMD of blackgram in Andhra Pradesh are closely related to MYMIV (old world geminiviruses) than MYMV (New world geminiviruses).

The sequence analysis of six isolates using SDT v1.2 revealed that YMV-GN and YMV-DES shared 99.58% homology which is the highest among the tested isolates whereas least homology of 94.85% was found between YMV-PR and YMV-WG. Whereas the two weed species YMV-DES and YMV-ABEL shared an identity of 99.30% at nucleotide level. The isolates collected from crop species *i.e.*, YMV-KR, YMV-GN, YMV-WG, YMV-PR shared a nucleotide similarity of 94.85 - 97.77%. Reddy *et al.* (2015) reported two variants of MYMIV infecting blackgram in Andhra Pradesh based on CP gene homology. Naimuddin *et al.* (2011) elucidated the causal agent associated with YMD of *V. mungo* var. *silvestris*, as MYMIV based on CP gene homology of more than 97% at nucleotide level and 99% at amino acid level. Usharani *et al.* (2004) studied MYMIV infecting soybean in Tamil Nadu and found that CP region has more than 96% homology with MYMIV isolates

and less than 80% with MYMV isolates. The earlier studies in Southern part of India on different isolates of YMV infecting blackgram, greengram and cowpea revealed no variation among MYMV isolates. However, YMV infecting horsegram was found similar to HgYMV which could be deciphered using CP gene. The study disclosed the efficiency of CP gene in early detection of YMV infection of pulses by MYMV and HYMV (Maheshwari *et al.*, 2014).

Chaitanya *et al.* (2020) reported the presence of both the species of begomoviruses *i.e.*, MYMIV and MYMV in Andhra Pradesh where MYMIV was found predominant compared to MYMV which are contradictory to the present results wherein, only MYMIV is reported in all the districts surveyed. These results suggests that MYMIV has replaced MYMV due to evolutionary changes.

## CONCLUSION

Previous survey reports for YMD in Andhra Pradesh revealed the prevalence of MYMV and MYMIV, but in the present study causal organism for the YMD was reported as MYMIV in all four surveyed districts. The molecular characterization of different isolates revealed that MYMIV population in Indian subcontinent is highly conserved irrespective of hosts and locations based on CP gene sequences. The variation in the incidence level might be due to stage of the crop at which infection occurred, variety grown, cropping system, cropping pattern and the management practices followed by the farmers. Further research on YMD management should be based on the development of resistant varieties using the genetic information of MYMIV blackgram isolates from various districts to develop coat protein mediated resistance due to its highly conserved nature over the years.

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